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Spatial structure of cyclosporin A and insight into its flexibility

S.V. Efimov [a], F.Kh. Karataeva [a], A.V. Aganov [a], S. Berger [b], V.V. Klochkov [a,*]

[a] Institute of Physics, Kazan Federal University, Kremlevskaya 18, Kazan 420008, Russia
[b] Institute of Analytical Chemistry, Leipzig University, Linnéstraße 3, Leipzig D-04103, Germany

HIGHLIGHTS

- Structures of cyclosporin A in $CHCl_3$ and in complex with SDS micelles are determined.
- Thermodynamic parameters of conformational exchange in $CHCl_3$ are found.
- Residual dipolar couplings and NOE are used as a source of structural information.

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ABSTRACT

The molecule of immunosuppressant drug cyclosporin A (CsA) exhibits different properties when dissolved in different media. In apolar solvents it is stabilized by intramolecular hydrogen bonds, but there also exist some less populated conformations. Existence of minor forms is clearly seen from ^{1}H NMR spectra.

Using nuclear Overhauser effect (NOE) spectroscopy and analysis of residual dipolar couplings, we obtained data on the molecular structure of the dominant conformers. Based on these data, the spatial structure of the main conformer of cyclosporin in chloroform was determined by molecular dynamics simulation. The kinetics of exchange between the major and minor forms was also studied. Energy barrier ($\Delta G^{\ddagger}$) between the two states is 81 ± 2 kJ/mol. The conformation of CsA in complex with sodium dodecyl sulphate micelles was determined from NOE data.

Use of independent structural data improves the reliability of the simulated results. The structure of the minor forms, which exist in organic solvents and also in micellar solution, cannot be assessed by means of nuclear magnetic resonance. Spectroscopic and thermodynamic parameters, however, point to their certain properties. In particular, the minor conformer of CsA in chloroform differs from the main one by a peptide bond (in *cis*- rather than *trans*-conformation) in the region of residues from 4 to 7.

1. Introduction

Spatial structure of molecules plays a crucial role in their biochemical function. The catalytic centre of an enzyme possesses a specific conformation. It should match exactly the shape of target molecules. Establishment of a proper structure during the protein folding process may be sometimes slowed down by relatively difficult steps, involving *cis–trans* isomerization of certain peptide bonds [1]. Erroneous folding can lead to dramatic consequences, one of the most well-known results being prionic diseases [2,3]. Sometimes, however, it is the unfavourable conformation of a molecule that makes it working. Studies of a cavity mutant of T4 lysozyme, L99A, showed that its cavity is inaccessible to ligands (substituted benzenes), but still binds them via an excited short-living state of low population [4]. Similarly, *cis-to-trans* transformation of the Mle^9–Mle^{10} peptide bond is a necessary step in binding of CsA to cyclophilin, and it is rather slow (k_{obs} = (7.6 ± 0.02) × 10^{-3} s^{-1} in aqueous medium [5]).

Cyclosporin A, cyclo (-Bmt-Abu-Sar-Mle-Val-Mle-Ala-D-Ala-Mle-Mle-Mva-) (see the scheme in Fig. 1), acts through formation of a complex with the 18-kDa protein cyclophilin (Cyp18). This protein is ubiquitous in many types of cells and exhibits Xaa-Pro *cis–trans* isomerase activity [6]. The complex CsA–Cyp18 inhibits calcineurin (Ca^{2+}-calmodulin activated serin–threonin phosphatase), and thus breaks the signal pathway which provides the reaction of T-cells to antigens [7–9]. Conformation of cyclosporin in the bound state differs from that in solution; in particular, the bond Mle^9–Mle^{10} adopts *trans*-conformation. These findings result from a continuous survey of cyclosporin structure on its own [10–12] and in complex with Cyp18 [13,14]. Attempts were made to synthesize cyclosporin analogues that inhibit calcineurin without binding to cyclophilin [5]. For this purpose, the primary sequence was modified in the region of residues 9–11, 1–3, responsible for CsA–Cyp18

* Corresponding author.
E-mail address: Vladimir.Klochkov@ksu.ru (V.V. Klochkov).

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